



Review of USB FFPW PhD thesis

First name(s), surname, titles of the PhD student: Ruoyi Hao, Ph.D.	First name(s), surname, titles of supervisor: Assoc. Prof. Dipl.-Ing. Jan Mráz, Ph.D.
Title of PhD thesis: Freshness and shelf-life of fish products	

REVIEWER:

Surname: Zemljič	Institution: University of Maribor Slovinsko
Name: Lidija Fras	
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Please describe your professional relationship to the PhD student: No relation	Please describe your field of expertise: Materials, functionalization, packaging, active coatings, modification, colloidal and surface chemistry

QUESTIONNAIRE

Originality, scientific importance, perspectives and impacts of results presented in the PhD thesis for basic and/or applied research

Evaluate competitiveness of the PhD thesis in the international context and compare its level with the current state of the art in the field (**extent ¼ – ½ page**):

The dissertation argues that fish gel food has great potential. However, the properties and stability of fish gel food are highly dependent on the freshness and quality of the fish muscle used for its preparation. The lipids, hemoglobin, metal ions, and heat-resistant enzymes in fish muscle can cause lipid and protein oxidation and muscle autolysis, resulting in deterioration of gel properties and shortening of shelf life. Therefore, it is important to find solutions to control metabolism or changes in fish muscle to provide fish proteins with excellent gelation properties and stability for gel food production.

The PhD **thesis is dedicated to this topic**, which is very extensive and complex, while it is dedicated to filling several gaps that exist in the preservation of fish food and the traceability of its freshness, clarifying:

- How the innovative culture system affects the nutrient pattern, spoilage process and freshness of carp.
- What indicator can be used to mark the freshness of n-3 carp and what are their limits.
- How to understand the role of microorganisms in fish freshness, and how can a good



preservation strategy be developed for using EO-based preservation methods to influence microbial spoilage and maintain freshness of cold-stored fish.

- How to improve the freshness and properties of fish gel products by physical and chemical preservation methods to consider the safety and needs of consumers and also to be environmentally friendly.

All these problems were clarified by the scientific work of candidate Ruoyi Hao, who developed new approaches:

- Demonstrating the differences between traditionally farmed carp and omega-3 carp in terms of potential nutrients, post-mortem freshness and shelf life; clarifying whether and how the patented farming system could affect the freshness and shelf life of carp.
- To analyze the chemical, physical and microbial characteristics of n-3 carp coated with EOs during cold storage to find a reliable freshness indicator; to get a good understanding of the effects of husbandry system and EO treatment on carp freshness.
- To study the spoilage microorganisms in cold stored fish and their relationships with typical freshness indicators, e.g. TVBN, TVC and nucleic acid.
- To understand the influence of preservation methods based on EOs on the freshness and shelf-life of cold stored fish, in order to provide clues for the development of appropriate preservation strategies by combining EOs with other technologies.
- To develop smart strategies to preserve the freshness and properties of fish gel food using phenolic compounds and ultrasonic treatment by improving protein cross-linking.

In summary, research filled the gap in the nutritional composition of Czech patented omega-3 carp; provided scientific data on the spoilage process, indicators of freshness and shelf life of cold stored omega-3 carp; added to the knowledge on the influence of spoilage microbes in cold stored fish by preservation methods based on EO, where sensory acceptability is not taken into account; developed a strategy for the use of EO emulsion coating to improve the freshness of carp; developed an intelligent strategy to control the oxidation of fish muscle protein to obtain good jelly properties.

Such a complex study has not been done before and is a novelty that gives Phd a rather high competitiveness. The excellent scientific work of the candidate is also reflected in the rather extensive publications in journals of high impact factor. The dissertation represents an important added value, seen not only in new scientific knowledge but also in the potential applicability for food/fish producers to improve the stability and shelf life of fish, taking into account safety and consumer requirements.



Elaboration of the PhD thesis, objectives of the work and deliverables

Evaluate the overall level of elaboration of the PhD thesis (structuring of the main text, comprehensibility, logicity of the chapters and their ordering) and the originality of the selected approaches to solve the objectives; evaluate publications and whether the results described correspond to objectives of the PhD thesis (extent ¼ – ½ page):

The main text is well structured and chapters follow the logicity. Chapter 1 gives a general introduction with an explanation of the main problem, how it is solved by the PhD student and where the study goes beyond the state of the art.

This is followed by Chapter 2, which describes the post-mortem quality changes of common carp (*Cyprinus carpio*) during cold storage in two culture systems. Chapter 3 provides a critical review of the use of essential oils against spoilage of chilled stored fish, including a quantitative meta-analysis. Chapter 4 continues with the development of an essential oil-based coating and its preservative effect on carp. Chapter 5 presents the influence of dose on the role of gallic acid in mediating the gelling properties of oxidatively stressed myofibrillar protein from Japanese lake fish. Chapter 6 examines how ultrasound treatment altered the effect of gallic acid on the properties of myofibrillar fish protein, and Chapter 7 provides a general discussion of all major issues and lists the main conclusions. Most of the chapters are described by scientific papers published in high impact factor journals.

For example, the second chapter lists the following article: (1) Hao, R.Y., Pan J., Khalili Tilami S., Shah B.R., Mráz J., 2020. Post-mortem quality changes of common carp (*Cyprinus carpio*) during chilled storage from two culture systems. *Journal of the Science of Food and Agriculture* 101, 91-100. Chapter 3 is presented through article of (2): Hao, R.Y., Roy, K., Pan, J., Shah, B. R., & Mraz, J. (2021). Critical review on the use of essential oils against spoilage in chilled stored fish: A quantitative meta-analyses. *Trends in Food Science & Technology*, 111, 175-190. Chapter 4 contains article by (3): Hao, R.Y., Shah, B. R., Sterniša, M., Možina, S. S., & Mraz, J. (2021). Development of essential oil-emulsion based coating and its preservative effects on common carp. *LWT - Food Science and Technology*. Accept. In Chapter 5, contribution (4) by: Pan J.F., Lian H.L., Jia H., Hao R.Y., Wang Y.J., Ju H.P., Li S.J., Dong X.P., 2020. Dose affected the role of gallic acid on mediating gelling properties of oxidatively stressed Japanese seerfish myofibrillar protein. *LWT - Food Science and Technology* 118, 108849 is presented, while Chapter 6 presents the fifth (5) article: Pan, J.F., Lian H.L., Jia H., Li S.J., Hao R.Y., Wang Y.J., Zhang X.N., Dong X.P., 2020. Ultrasound treatment modified the functional mode of gallic acid on properties of fish myofibrillar protein. *Food Chemistry* 320, 126637.

All articles are of high quality and are graded A1 or A2. All together provide a structured and detailed as well as logical view of the hypothesis, objectives and proven results with new solutions. All results meet the objectives with very clear consistency and clearly relate to the corresponding specific article.



OVERALL COMMENTARY ON THE PhD THESIS

Please write in the box specific comments concerning the PhD thesis in extent of 1-2 pages:

The following conclusions arise from this dissertation:

- Omega-3 carp from the patented farming system showed a slightly positive effect on muscle stability of carp and a slightly longer shelf life than carp from the traditional farming system in the Czech Republic. The patented system could be used to produce carp with high n-3 PUFA and n-3/n-6 ratio without affecting storage stability.
- Four SSOs, i.e., *Pseudomonas* spp, *Shewanella* spp, lactic acid bacteria, and Enterobacteriaceae, are common spoilage microbes in cold stored fish.
- Six EOs, namely, Citrus, Mentha, Origanum, Thymus, Zataria and Zingiberaceae EOs were able to inhibit TVC in fish. However, only Origanum, Zingiberaceae and Thymus EOs showed complete efficacy on the above four SSOs.
- To ensure a high preservative effect on the freshness and shelf life of cold stored fish, a suitable combination of preservation methods is required, especially hurdle technology, active films, nanoemulsions and special packaging.
- The incorporation of 1% allspice, thyme and oregano EO emulsion into an alginate coating can adequately preserve carp in cold storage without causing unacceptable sensory characteristics. Protein oxidation can be well manipulated by an appropriate dose of gallic acid and ultrasonic treatment to alter the desired protein properties and improve the jelly properties of the fish product.

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The dissertation is well prepared with clear statements and overviews in each chapter.

The general introduction provides an overview of the importance of fish food, fish muscle deterioration and its mechanisms, enzymatic autolysis, oxidation and microbial spoilage. Furthermore, the overview of freshness indicators for fish or fish products is described, such as common freshness indicators: sensory indicators, physical indicators, chemical indicators, microbiological indicators. Reference is made to the recommended limits in this chapter. Effective preservation methods are also explained: physical, biological and chemical methods. The research objectives are clearly defined with appropriate indicators. The research results are defined by the above-mentioned research articles (1-5) in a logical order, with the general discussion making clear the main results related to each article. Thus, autolysis of fish, microbial spoilage of fish, fish freshness indicators and their limitations are discussed using a complex methodology to obtain comprehensive results related to the corresponding article. It is important to summarise the results of EOS for the preservation of cold-stored fish and apply them in practise. The new findings on improving the stability of fish gelatin by GA are presented, again with reference to appropriate articles as contained and given in the thesis. Finally, the conclusions are given in an understandable and concise manner, with the novelty behind them understood.

The results are of scientific and economic practical importance, increase credibility for the user and are an innovative contribution to science in the field of fish conservation strategies. The innovative scientific contribution is evident in the 5 articles published in high impact



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factor journals involving complex methodology and in-depth discussion.

The PhD is innovative and interdisciplinary, covering following areas: food, biotechnology, microbiology, materials and the social aspect (consumer perspective).

FINAL RECOMMENDATION

- ☒ **PhD thesis can be recommended for defence**
- ☐ **PhD thesis can be recommended for defence with reservations**
- ☐ **PhD thesis cannot be recommended for defence**

Maribor, 21.12. 2021
Date and place

Lidija Fras Zemljič
Name and signature